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# Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

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# Application No. Applicant(s) 09/923 870 PALSSON, BERNHARD Office Action Summary Examiner Art Unit RUSSELL S. NEGIN 1631 -- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --Period for Reply A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS. WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION. Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication. If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication. Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b). Status 1) Responsive to communication(s) filed on 18 June 2009. 2a) This action is FINAL. 2b) This action is non-final. 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11, 453 O.G. 213. Disposition of Claims 4) Claim(s) 49-52.56-60.64 and 68-83 is/are pending in the application. 4a) Of the above claim(s) is/are withdrawn from consideration. 5) Claim(s) \_\_\_\_\_ is/are allowed. 6) Claim(s) 49-52.56-60.64 and 68-83 is/are rejected. 7) Claim(s) \_\_\_\_\_ is/are objected to. 8) Claim(s) are subject to restriction and/or election requirement. Application Papers 9) The specification is objected to by the Examiner. 10) The drawing(s) filed on is/are; a) accepted or b) objected to by the Examiner. Applicant may not request that any objection to the drawing(s) be held in abevance. See 37 CFR 1.85(a). Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d). 11) The oath or declaration is objected to by the Examiner, Note the attached Office Action or form PTO-152. Priority under 35 U.S.C. § 119 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f). a) ☐ All b) ☐ Some \* c) ☐ None of: Certified copies of the priority documents have been received. 2. Certified copies of the priority documents have been received in Application No. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)). \* See the attached detailed Office action for a list of the certified copies not received.

1) Notice of References Cited (PTO-892)

Paper No(s)/Mail Date 6/18/09; 7/16/09.

Notice of Draftsperson's Patent Drawing Review (PTO-948)
 Information Disclosure Statement(s) (PTO/SB/CS)

Attachment(s)

Interview Summary (PTO-413)
 Paper No(s)/Mail Date.

6) Other:

5) Notice of Informal Patent Amilication

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#### DETAILED ACTION

#### Comments

Applicants' request for reconsideration in the communication filed on 18 June 2009 is acknowledged.

Claims 49-52, 56-60, 64 and 68-83 are pending and examined in the instant Office action.

## Information disclosure statements

The information disclosure statements filed on 18 June 2009 and 16 July 2009 have been considered. It is noted that the references stricken through on the information disclosure statement of 18 June 2009 have not been provided by applicant. It is also noted that reference #285 of Schilling (2000) only disclosed the first two pages of the document. Citations 369, 371, 376, and 383 do not have dates. A date and place of publication are required under 37 CFR 1.98. Also, with regard to some of the URL citations, the complete citation is not filed. For example, citation 369 cites the SWISS-PROT database, citation 373 cites the EcoCyc database, citation 376 cites the KEGG database, citation 379 cites the MPW database, citation 382 cites the Genbank database, citation 383 cites the NCBI genome database, and citation 384 cites the LocusLink database; however, while these databases are cited on the information disclosure statement, entire copies of the databases are not provided as required. It appears that applicant may be attempting to make specific webpages, obtained from a

database, of record. Is this is the case, then the citations are incorrect as they refer to the entirety of each database.

It is also noted that the documents on the information disclosure statement of 16 July 2009 are all duplicates of articles on the information disclosure statement of 18 June 2009. All citations on the IDS of 16 July 2009 have been crossed out to avoid duplication upon printing.

Applicant is advised that the date of any re-submission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the statement, including all certification requirements for statements under 37 CFR 1.97(e). See MPEP § 609.05(a).

## Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The following rejection is reiterated:

35 U.S.C. 103 Rejection #1:

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Claims 49-52, 56-60, and 68-83 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pramanik et al. [Biotechnology and Bioengineering, volume 56, 1997, pages 398-421] in view of Blattner et al. [Science, volume 277, 1997, pages 1453-1469] in view of Kunst et al. [Reviews in Microbiology, 1991, volume 142, pages 905-912].

Claim 49 is drawn to a method performed in a computer of simulating a metabolic capability of an in silico strain of a microbe. The method comprises obtaining a plurality of DNA sequences comprising most of the metabolic genes in a genome of the microbe, to produce an in silico representation of a microbe. The method additionally comprises determining open reading frames of genes of unknown function in the microbe in the plurality of DNA sequences. The method additionally comprises assigning a function to proteins encoded by the open reading frames by determining the homology of the open reading frames to gene sequences encoding proteins of known function. The method additionally comprises determining which of the open reading frames correspond to metabolic genes by determining if the assigned function of the proteins is involved in cellular metabolism. The method additionally comprises determining substrates, products, and stoichiometry of the reaction for each of the gene products of the metabolic genes. The method additionally comprises producing a genome specific stoichiometric matrix of the microbe produced from said substrates, products and stoichiometry into a stoichiometric matrix. The method additionally comprises determining a metabolic demand corresponding to a biomass composition of the microbe. The method additionally comprises calculating uptake rates of metabolites of the microbe. The method additionally comprises combining the metabolic demands and

the uptake rates with the stoichiometric matrix to produce an in silico representation of the microbe. The method additionally comprises incorporating a general linear programming problem to introduce an in silico strain of the microbe. The method additionally comprises performing a flux balance analysis on the in silico strain. The method additionally comprises providing a visual output to a user of said analysis that simulated a metabolic capability of said strain predictive of the microbe's phenotype.

Claim 57 is drawn to a method performed in a computer for simulating a metabolic capability of an in silico strain of a microbe analogous to the method of claim 49.

Claims 50 and 58 are further limiting wherein the microbe is question is E. coli.

Claims 51, 59, 68, 70-72, 75-76, 78-80, and 83 are further limiting wherein

cellular metabolism comprises central metabolism, carbohydrate assimilation, lipid and fatty acid metabolism and nucleotide metabolism.

Claims 52 and 60 are further limiting wherein the assigning function comprises performing BLAST.

The study of Pramanik et al. investigates the stoichiometric model of E. coli metabolism, as stated in the abstract:

A stoichiometric model of metabolism was developed to describe the balance of metabolic reactions during steady state growth of Escherichia coli on glucose (or metabolic intermediates) and mineral salts. The model incorporates 153 reversible and 147 irreversible reactions and 289 metabolites from several metabolic data bases...

Consequently, Pramanik et al. studies many metabolic reactions that take place within E. coli (i.e. see the list in Appendix A on pages 411-417). Equations 1 and 2 on

page 399 denote the flux model metabolism via a mass balance on E. coli wherein the matrix S is a matrix of stoichiometric coefficients relevant to the equations.

Table VII on page 405 of Pramanik et al. lists the upper and lower bounds of metabolite uptake and secretion, and the text under this table describes sensitivity of each type of metabolism (i.e. aerobic or anaerobic) due to biomass composition. This biomass and energy requirement (i.e. demand) is elaborated further under "Biomass and energy requirements" in column 2 on page 399 of Pramanik et al.

Again, equations 1 and 2 on page 399 of Pramanik et al. represent a mass balance on the metabolites of E. coli which combines the metabolic demands, uptake rates and the stoichiometric matrix to produce an in silico representation of functions of said microbe.

Page 403 of Pramanik et al., under "Sensitivity Analysis," lists a general linear programming algorithm for solving equations 1 and 2 on page 399 of Pramanik et al.

Figure 3 on page 406 of Pramanik et al. illustrates a flux balance analysis on the in silico strain of E. coli and provides visual output to a user that simulates a metabolic capability of the strain.

However, Pramanik et al. does not teach obtaining a plurality of DNA sequences comprising most of the metabolic genes in an genome, determining open reading frames of these genes, assigning functions to the proteins encoded by the open reading frames, and determining which of said open reading frames correspond to metabolic genes. Additionally, Pramanik et al. does not teach determining open reading frames of

genes having an unknown function and assigning a function to their encoded products based on homology to proteins in a different organism.

The study of Blattner et al. maps the complete genome sequence of Escherichia coli K-12.

The final full paragraph of column 3 on page 1454 of Blattner et al. states, "The genome of E. coli, diagrammed in Fig. 1, consists of 4,639,221 bp of complex DNA."

Consequently, Figure 1 on page 1465 of Blattner et al. illustrates an *in silico* map of the complete genome of E. coli.

The first full paragraph of column 1 on page 1454 of Blattner et al. describes the annotation process of identifying ORFs in genes constituting operons, regulatory sites, mobile genetic elements, and repetitive sequences in the genome, assigning and suggesting functions, and relating the E coli. sequence to other organisms. The second full paragraph of column 1 on page 1454 states:

Functions of previously known E. coli proteins were collected from the GenProtEC and EcoCyc database. The function of new translated sequences was inputted from sequence similarity.

Consequently, functions are assigned to proteins by determining similarities (i.e. homologies) to proteins of known function.

The result of assigning functions to proteins is Table 4 on page 1459 of Blattner et al. wherein metabolic genes are classified as such in their specific metabolic classes listed in Table 4 of page 1459 of Blattner et al. One of the classes listed is nucleotide synthesis and metabolism.

The first full paragraph of column 2 on page 1454 of Blattner et al. describes the use of BLAST in assigning function to proteins.

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Pramanik et al. and Blattner et al. do not teach determining open reading frames of genes having an unknown function and assigning a function to their encoded products based on homology to proteins in a different organism.

The review of Kunst et al. studies the project of sequencing the entire Bacillus substilis genome. Applicant states in the paragraph bridging columns 1 and 2 on page 905 of Kunst et al.:

At the first level of analysis, the DNA sequence will lead to a complete catalogue of putative protein sequences. These are likely to fall into one of 3 categories: (1) those whose functions are known, (2) those which show similarities with proteins identified in other organisms and which may have similar though not necessarily identical functions in B. substilis, and (3) those, probably the majority, whose function is unknown at present....

Consequently, the "unknown" functions of protein sequences are identifiable by homology comparison with corresponding sequences in other organisms. Such analysis allows the identification of function of two proteins with unknown function (see abstract of Kunst et al.).

Kunst et al. continue in the last paragraph of page 905:

It will be of great interest to compare the sequence of the *B. substillis* genome with that of *E. coli....* These organisms diverged about 1.2 billion years ago... It will be interesting to examine the conservation of "core" genes responsible for general metabolism.

Consequently, Kunst et al. express great interest in comparing the genomes of *E. coli* and *B. subtilis* as they pertain to metabolism.

Here is a chart illustrating where each of the species in claims 68-83 are found in the above prior art references:

Central metabolism	Claims 68, 76	Kuntz et al., introduction
Amino acid metabolism	Claims 69, 77	TCA cycle in Pramanik et al.

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Nucleotide metabolism	Claims 70, 78	TCA cycle in Pramanik et al.
Fatty acid metabolism	Claims 71, 79	Pramanik et al., page 339, c2
Lipid metabolism	Claims 72, 80	Pramanik et al, page 339, c2
Vitamin/co factor synthesis	Claims 73, 81	Appendix A, Pramanik et al.
Energy and redox	Claims 74, 82	Appendix A, Pramanik et al.,
Carbohydrate assimilation	Claims 75, 83	Appendix A, Pramanik et al. and glycolysis

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the stoichiometric model of E. coli metabolism as taught by Pramanik et al. by use of the complete genome sequence of Blattner et al. wherein the motivation would have been that by knowledge of the full genome of E coli, not only can metabolism be further analyzed, but also knowledge of the entire sequence of E. coli enables global approaches to understanding biological function in living cells and has led to new ways of looking at the evolutionary history of bacteria [see first paragraph of introduction on page 1453] It would have been further obvious to modify the stoichiometric model of E. coli metabolism as taught by Pramanik et al. and the complete E. coli genome structure in Blattner et al. by use of the comparison of E. coli and B. substilis metabolism genes and proteins to determine function of unknown proteins as in Kunst et al. wherein the motivation would have been that such homology comparisons allow for an analysis of the differences in evolution between the two different, but related, organisms to result in improvement in industrial applications [see

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last sentence on page 905 of Kunst et al. and first paragraph on page 906 of Kunst et al.].

### Response to Arguments:

Applicant's arguments filed 18 June 2009 have been fully considered but they are not persuasive.

Applicant first argues on page 9 of the Remarks that Pramanik et al. teaches only known genes encoding proteins with known biological activity. This argument is not persuasive and is the rationale by which the reference of Kunst et al. is used in the instant obviousness prior art rejection.

Applicant further argues that there is no motivation to combine the reference of Blattner et al. with Pramanik et al. This argument is not persuasive because as explained above, usage of the entire genome of E. coli not only provides further analysis of metabolism, but also permits new ways at looking at the evolutionary history of bacteria.

Applicant further argues that there is no motivation or reasonable expectation of success in combining Kunst et al. with Pramanik et al. and Blattner et al. (i.e. incorrect information would be incorporated into the model as a result of the combination of references). These arguments are not persuasive because, as discussed above, the comparisons of the homologies between two different, but related organisms results from the combination of Kunst et al., Pramanik et al., and Blattner et al. Furthermore, there would have been a reasonable expectation success in combining the

stoichiometric matrices corresponding to known biological functions in Pramanik et al. and Blattner et al. with the set of sequences of Kunst et al., wherein a majority of which have unknown biological function, because the functions and structural stoichiometries of the relations in Pramanik et al. are analogous to the kinetics of homologous organisms as taught in the introduction of Kunst et al.

## The following rejection is reiterated:

## 35 U.S.C. 103 Rejection #2:

Claim 64 is rejected under 35 U.S.C. 103(a) as being unpatentable over

Pramanik et al. in view of Blattner et al. in view of Kunst et al. as applied to claims 49-52
and 56-60 above, and further in view of Xie et al. [TIBTECH, 1997, volume 15, pages
109-113].

Pramanik et al., Blattner et al., and Kunst et al. make obvious a method of simulating a metabolic capability of an in silico strain of a microbe by simulating metabolism within E. coli, as discussed above.

Pramanik et al., Blattner et al., and Kunst et al. do not teach calculation of uptake rates by measuring depletion of substrate from the growth media.

The study of Xie et al. studies integrated approaches to the design of media and feeding strategies for fed-batch cultures of animal cells.

Column 2 on page 109, lines 3-7 of Xie et al. states:

Obviously, a high viable cell density maintained for a long time is required to maximize product concentration. However, this mainly depends upon the composition of the medium employed.

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Consequently, the composition of the growth medium and its depletion over time affects the growth of the cells.

The motivation of the study of Xie et al. is that by knowing this fact, better compositions for culture media can be designed (i.e. see page 110 of Xie et al.)

It would have been obvious to someone of ordinary skill in the art at the time of the instant invention to modify the metabolism studies of E. coli of Pramanik et al.

Blattner et al., and Kunst et al. by use of the nutrient depletion studies of Xie et al. wherein the motivation would have been by knowing how nutrients are depleted in order to facilitate cell growth, stronger media can be designed to enable better growth of the cells in the cellular media [see page 110, column 1-2 under "Motivation for medium design," and "Design of culture environment."] There would have been a reasonable expectation of success in applying the animal cell study of Xie et al. to the bacterial studies of Pramanik et al. and Blattner et al. because when all cells are cultured, whether animal or bacterial, the cells need nutrients to survive, and as a result, all species of cells deplete their culture media of these nutrients.

# Response to Arguments:

Applicant's arguments filed 18 June 2009 have been fully considered but they are not persuasive.

Applicant argues that the reference of Xie et al. does not overcome the alleged deficiencies of the combination of references in the first 35 U.S.C. 103 rejection. This

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argument is not persuasive because this combination of references teaches all of the required limitations in the instantly rejected claims.

### Double Patenting

# The following rejection is reiterated from the previous Office action:

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to

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be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 49-52, 56-60 and 64 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 26-28, 30, 32, 35-36, and 39-41 of copending Application No. 11/980,199. Although the conflicting claims are not identical, they are not patentably distinct from each other because the set of claims of the reference describes the same *in silico* process for open reading frames, determining the metabolic capability of a strain of a microbe. For instance, the steps of claim 30 of '199 correspond with the steps of instant independent claim 49. Each claim has the steps of obtaining DNA sequences, determining ORFs, assigning functions to proteins, determining which open reading frames correspond to metabolic genes, determining a stoichiometric balance on relevant metabolic reactions, determining metabolic demand, calculating uptake rates, combining metabolic demands with uptake rates, incorporating general linear programming, performing a flux balance, and providing visual outputs to a user. Additionally claim 41 of the reference has steps that correspond with the steps of instant independent claim 57.

This is a <u>provisional</u> obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

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# Response to Arguments:

Applicant requests that this rejection be held in abeyance.

### Declarations

The declarations under 37 CFR 1.132 filed 18 June 2008 are insufficient to overcome the rejection of claims 49-52, 56-60, 64 and 68-83 based upon 35 U.S.C. 103 as set forth in the last Office action. It is noted that while the Remarks of applicant acknowledge three declarations- on each from Bernhard Palsson, Jay Keasling, and Dr. Nielsen, only the declarations from Bernhard Palsson and Jay Keasling have been received by the Office.

With regard to the declaration of Palsson, applicant reiterates the arguments from the Remarks regarding lack of a motivation and a reasonable expectation of success in combining the references. Again, these arguments are not persuasive because the comparisons of the homologies between two different, but related organisms results from the combination of Kunst et al., Pramanik et al., and Blattner et al. Furthermore, there would have been a reasonable expectation success in combining the stoichiometric matrices corresponding to known biological functions in Pramanik et al. and Blattner et al. with the set of sequences of Kunst et al., wherein a majority of which have unknown biological function, because the functions and structural stoichiometries of the relations in Pramanik et al. are analogous to the kinetics of homologous organisms as taught in the introduction of Kunst et al.

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Palsson also argues that there was long felt need in the art as a result of the colleague (James Bailey) stating that if the model worked, it would be a breakthrough. In response to these secondary considerations for lack of obviousness and the Remarks of James Bailey, Palsson does not supply direct evidence in the scientific or patent literature (i.e. formally distinguishing, with specific reasoning, at least some of the references from the hundreds listed in the information disclosure statements) such that James Bailey's assertions are supported by more people of ordinary skill in the art in the field. While paragraph 16 of the declaration cites some important studies in the field, Palsson does not point to where, in any of the studies, the determination of growth rates and pathway rates without knowledge of kinetics is a breakthrough.

The declaration of Jay Keasling reiterates the unexpected results of metabolic simulation of genes with unknown function using homologus genomes with known functions. This argument is not persuasive because Kunst et al. teaches this same nexus in the introduction on pages 905-906. Specifically, Kunst et al. teaches that structures and functions of sequences with unknown function in an organism can be determined by comparison with genes in an other organism with similar homology. Kunst et al. even states that such comparisons result in improvement in the expression of enzymes and metabolites in industrially important organisms [see first paragraph, page 906 of Kunst et al.]

#### Conclusion

No claim is allowed.

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THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the central PTO Fax Center. The faxing of such pages must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CFR § 1.6(d)). The Central PTO Fax Center Number is (571) 273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Negin, whose telephone number is (571) 272-1083. The examiner can normally be reached on Monday-Friday from 7am to 4pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisor, Marjorie Moran, Supervisory Patent Examiner, can be reached at (571) 272-0720.

Information regarding the status of the application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information on the PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/RSN/ Russell S. Negin 22 September 2009

/Marjorie Moran/ Supervisory Patent Examiner, Art Unit 1631